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ARTICLE

Effect of a single dose of subcutaneous meloxicam before knife castration alone or combined with hot-iron branding on scrotal healing, inflammatory response, and behaviour in 2-mo-old beef calves over 42 d post procedure[1]

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Abstract: The objective of this study was to evaluate the effect of a single subcutaneous injection of meloxicam on scrotal healing, inflammatory response, and behaviour in castrated or castrated and branded beef calves for 42 d post procedure. Seventy-two 2-mo-old Angus crossbred bull calves were used to assess the effects of procedure (Trt): (1) sham control, (2) knife castration, and (3) knife castration and hot-iron branding; and pain mitigation (Med): (1) nonmedicated and (2) medicated with meloxicam according to a 3 × 2 factorial design. Body weight, scrotal circumference (SC), and healing scores were collected weekly until day 42. Blood samples were collected weekly until day 42 to assess haptoglobin, serum amyloid-A, and complete blood cell count. Hair was collected on day −1 and day 42 to assess cortisol concentrations. Lying and standing behaviour were recorded for 42 d, whereas pain-related behaviours were recorded on days 7, 15, 22, 29, and 34 post procedure. The inflammatory response (SC) and duration of standing was greater ($P < 0.05$) in calves castrated and branded than those only castrated. However, meloxicam did not reduce inflammation or improve wound healing in either castrated or castrated and branded calves.

Key words: beef, castration, branding, inflammatory response.

Résumé : L'objectif de cette étude était d'évaluer l'effet d'une seule injection sous-cutanée de méloxicam sur la guérison scrotale, la réponse inflammatoire et le comportement chez les veaux de bœuf castrés ou castrés et marqués lors des 42 j après la procédure. Soixante-douze veaux mâles Angus croisés âgés de 2 mois ont été utilisés pour évaluer les effets de la procédure (Trt) : (1) témoin négatif, (2) castration au couteau et (3) castration au couteau et marquage au fer chaud; et l'effort pour mitiger la douleur (Méd) : (1) sans médicament et (2) avec médicament méloxicam selon un plan expérimental factoriel 3 × 2. Le poids corporel, la circonférence scrotale (SC − « scrotal circumference ») et les cotes de guérison ont été collectés de façon hebdomadaire jusqu'au jour 42. Les échantillons de sang ont été collectés de façon hebdomadaire jusqu'au jour 42 pour évaluer le taux d'haptoglobine, de l'amyloïde A sérique et le compte complet des cellules sanguines. Le poil a été collecté aux jours -1 et 42 pour évaluer les concentrations de cortisol. Les comportements de position couchée et position debout ont été enregistrés pendant 42 j

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tandis que les comportements reliés à la douleur ont été enregistrés aux jours 7, 15, 22, 29 et 34 après la procédure. La réponse inflammatoire (SC) et la durée en position debout étaient plus grandes ($P < 0,05$) chez les veaux castrés et marqués que chez ceux castrés seulement. Par contre, le méloxicam n'a pas réduit l'inflammation ni amélioré la guérison de blessure autant chez les veaux castrés que chez les veaux castrés et marqués. [Traduit par la Rédaction]

Mots-clés : bœuf, castration, marquage, réponse inflammatoire.

Introduction

The most common painful procedures experienced by beef cattle in North America include castration, dehorning, and branding (Tucker et al. 2015). Castration of calves is known to be painful regardless of the method and the age it is performed (Robertson et al. 1994; Molony et al. 1995; Marti et al. 2017*a*; Meléndez et al. 2017*a*). Castration is typically performed in conjunction with other painful procedures such as dehorning, branding, and vaccination. To date, all published studies evaluating the combined effect of two painful routine management procedures have assessed castration in combination with dehorning (Schwartzkopf-Genswein et al. 2005; Ballou et al. 2013; Mosher et al. 2013; Sutherland et al. 2013). Hot-iron branding is a method used to permanently identify cattle, and it is still commonly used in countries where no electronic identification is required or in areas where producers comingle animals on community pastures. The few studies that have evaluated the welfare implications of hot-iron branding have reported several indicators associated with pain (Lay et al. 1992; Schwartzkopf-Genswein et al. 1997*a*, 1997*b*, 1997*c*; Tucker et al. 2014*a*, 2014*b*). For example, hot-iron branded calves had greater tail-flick, kick, and fall frequencies at the time of branding (Schwartzkopf-Genswein et al. 1997*a*), as well as greater heart rate (Lay et al. 1992) and plasma cortisol concentrations (up to 40 min post branding) (Lay et al. 1992; Schwartzkopf-Genswein et al. 1997*c*), than freeze or unbranded control calves. In addition, the use of a von Frey anesthesiometer indicated that branding wounds remain sensitive for at least 10 wk after branding (Tucker et al. 2014*a*, 2014*b*).

Broom and Johnson (2000) suggested that two noxious stimuli potentially generate greater physiological and behavioural responses than either one alone when performed at the same time. For example, Ballou et al. (2013) and Sutherland et al. (2013) observed an increase in plasma cortisol concentrations when castration and dehorning were combined compared with when they were done alone, and the behavioural responses were elevated by combining both procedures (Sutherland et al. 2013). In contrast, Mosher et al. (2013) did not find differences in plasma cortisol, when castration was performed alone or concurrently with dehorning; however, in this study, calves were acclimated by sham castration and sham dehorning the day before. To date, no published studies have evaluated the combination of castration and branding or their long-term effects (lasting more than 7 d post procedure) on indicators of pain, inflammation, or wound healing when the two procedures are combined.

The Canadian Codes of Practice for the care and handling of beef cattle (NFACC 2013) recommend the use of pain control when painful procedures such as castration or branding are performed. However, a recent Canadian survey of cow–calf producers found that only 4% of respondents used pain mitigation when castrating calves younger than 3 mo of age, and 4% used pain control during branding (Moggy et al. 2017).

Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) that has been recently tested to mitigate the pain of castration in weaned calves (Brown et al. 2015; Roberts et al. 2015; Meléndez et al. 2017*b*). Furthermore, the anti-inflammatory properties of oral meloxicam have been shown to reduce morbidity in weaned castrated beef calves suggesting that its effect may last several days after a single treatment (Coetzee et al. 2012). However, to date no studies have been conducted evaluating its efficacy in reducing long-lasting stress and inflammatory pain, inflammation, or healing beyond 1 wk post castration. Mintline et al. (2014) did not find a reduction in inflammation or improved rate of healing after the administration of a single dose of flunixin meglumine following castration in beef calves. In addition, there are very few published studies on the effects of subcutaneous (s.c.) meloxicam on mitigating pain in calves younger than 3 mo of age (Creutzinger et al. 2017; Meléndez et al. 2018*a*), or on multiple painful procedures conducted concurrently. The aim of this study was to evaluate long-lasting stress, inflammation, scrotal healing, and behaviour in 2-mo-old beef calves after castration alone or combined with branding, with or without s.c. meloxicam 7–42 d post procedure(s). Our hypotheses were that (1) the combination of multiple painful procedures would produce greater long-lasting stress and inflammatory pain, and delay healing associated with greater tissue damage, and (2) meloxicam would reduce post-surgical inflammation for several days post procedure(s) due to its long (22 h) plasma half-life.

Materials and Methods

All procedures described within this study were approved by the Animal Care Committee of the Lethbridge Research Centre (LRC), Agriculture and Agri-Food Canada, Lethbridge, AB, Canada (ACC1410), and University of Calgary Animal Care Committee (AC14-0159) according to the guidelines established by the Canadian Council on Animal Care (2009). This paper is part of a larger study evaluating the effect of a single

injection of s.c. meloxicam on indicators of pain after castration and branding in 2-mo-old beef calves in which Meléndez et al. (2018*b*) assessed the acute pain responses (up to 7 d post procedure), and the current study assessed the post-operative responses (between 7 and 42 d post procedure), such as scrotal healing, inflammatory response, and behaviour.

Animals, housing, and treatments

A total of 72 Angus crossbred bull calves were used to conduct a study using a completely randomized design in which calves were blocked by body weight (BW) and assigned according to a 3 × 2 factorial arrangement of treatments to evaluate the effect of procedure: (1) sham control (CN) in which calves were handled in a similar manner and for a similar amount of time as castrated animals including manipulation of the scrotum, and placement of a ambient temperature (cool) iron on the same location as calves that were branded; (2) knife castration (KN) in which calves were surgically castrated, and a cool brand was placed on the same location for a similar amount of time as the hot-iron branded group, and (3) knife castration and branding (KB) in which calves were surgically castrated, and a hot-iron brand was placed on the right rib of the calves; and the effect of pain mitigation: (1) nonmedicated calves (NM) injected with lactated ringer's solution (Lactated Ringer's Irrigation, Baxter Canada, Mississauga, ON, Canada) with the same volume as the medicated group and (2) medicated calves (M) injected with 0.5 mg kg^{-1} of BW of meloxicam (Metacam 20 mg mL^{-1}, Boheringer Ingelhein, Burlington, ON, Canada). Lactated ringer's solution and meloxicam were injected s.c. immediately prior to castration using a 1.6 mm × 25 mm needle in the neck of the calves. Surgical castration was performed by an experienced veterinarian using a Newberry knife (Syrevet Inc., Waukee, IA, USA) to make an incision in the scrotum and remove the testicles followed by the application (30–60 s) of a sterilized emasculator to sever and crush the spermatic cords. Branding was performed by an experienced LRC feedlot employee using an electric branding iron (size: 6.35 cm × 14.5 cm; temperature range: 163–204 °C, El-Toro electric brands, Sherwood Park, AB, Canada), which consisted of a number, a symbol, and a letter placed on the right rib. Calves were separated from their dams during the procedure and sampling times; distance between the outdoor handling area and home pens was between 34 and 200 m. During castration and branding, calves were restrained using a tip table (Calf Roper, Ram-Bull Ltd., Barons, AB, Canada), and all subsequent samples and BW were collected in a portable chute (Persons Livestock Equipment, Thedford, NE, USA). Calves were castrated for an average time of 1.1 ± 0.19 min, branded for 0.5 ± 0.18 min and sampled for 3.1 ± 2.75 min. All samples over the course of the study were collected at similar times in the morning.

Calves were born in April and kept as bulls. Cow–calf pairs were transported approximately 30 km from a local ranch to the LRC feedlot on 10 June 2015 and divided in two groups (36 calves per group) that were castrated and branded a week apart following the same procedures (group 1 on 24 June 2015, and group 2 on 30 June 2015). All calves were between 67 and 87 d of age at the time of the procedures.

After being assigned to either group 1 or 2, calves and cows were allocated to one of six pens (group 1: three pens of 36 m × 22 m; 12 cow–calf pairs per pen; group 2: three pens of 40 m × 27 m; 12 cow–calf pairs per pen) equally distributed by weight, where treatments were mixed within pens. Pens had wind break fencing on the north side, and also contained calf shelters (2.4 m × 3.6 m × 1.4 m) and deep straw bedding, which was used to keep the animals dry and clean. One hay bale feeder was placed in each pen to provide cows with alfalfa grass. Each pen had a water trough (0.8 m × 0.4 m × 0.5 m) to provide fresh water. Feed and water were offered ad libitum.

Measurements and sample collection

Body weight was collected on day −1, immediately prior to castration (day 0) and weekly thereafter until the end of the study. The experiment lasted 42 d, which was when the scrotum of the knife-castrated calves had healed. In addition, BW was collected at the time of weaning (day 90 from the beginning of the experiment).

On day −1 and 42, hair from the forehead of each calf was collected to measure hair cortisol concentrations, which is an indicator of long-lasting stress and might be a proxy for inflammatory pain. Hair was shaved from the forehead of each calf using clippers (Lister, Wahl Clippers Corp., Sterling, IL, USA) on day −1 to remove the existing hair and ensure that the cortisol deposited within the shaft of the regrowth (hair grows at 0.6–1 cm per month) (Comin et al. 2011) would best reflect the responses directly related to the procedures over a 42 d period post treatment. Although hair from the forehead is known to contain reduced concentrations of cortisol compared with other locations such as the tail switch, it was used for analysis in this study due to cleanliness of the area and ease of sample collection when calves were restrained in the squeeze chute. Hair samples were processed, and quantification of cortisol was determined using an immunoassay technique (Salimetric Assay Kit, State College, PA, USA) following Moya et al. (2013). The intra- and inter-assay coefficients of variability (CV) were 6.7% and 16.7%, respectively.

To evaluate the acute-phase response, a 10 mL blood sample was collected on day −1 and the day immediately prior to castration (day 0), and weekly thereafter until the end of the study via jugular venipuncture from all calves using a nonadditive tube (BD Vacutainer, Becton Dickinson Co., Frankin Lakes, NJ, USA) for the analysis of haptoglobin (Hp) and serum amyloid-A (SAA). Samples were centrifuged at 1500*g* at 4 °C for 15 min, and the serum were decanted and stored at

−20 °C until analysis. Haptogloblin assays were performed at the University of Guelph (Animal Health Laboratory, University of Guelph, Guelph, ON, Canada) as described by Marti et al. (2017*a*), and the interassay CV was 7.6%. An enzyme-linked immunosorbent assay (Tridelta Phase Serum Amyloid A, Tridelta Development Ltd., Maynooth, Kildare, Ireland) was used to analyze SAA. The intra- and inter-assay CV were 5.7% and 13.5%, respectively. Another 6 mL of blood was collected into a tube containing EDTA (BD Vacutainer, Becton Dickinson Co., Frankin Lakes, NJ, USA) on day −1 and the day immediately prior to castration (day 0), and weekly thereafter until the end of the study for complete blood cell count (CBC). The CBC was measured using a HemaTrueHematology Analyzer (Heska, Loveland, CO, USA) to determine the concentration of white blood cells (WBC), platelets, and neutrophil:lymphocyte ratio (N:L).

On day −1, immediately prior to castration (day 0), and weekly thereafter until day 42, maximum temperature of the scrotum (MST) and scrotal circumference (SC) were measured to evaluate inflammation. Images of the scrotal area were taken from behind the calf at a distance of 1 m according to Moya et al. (2014). The MST was measured using an infrared camera (Flir i60, Flir systems Inc., Burlington, ON, Canada), and processed with ThermCam QuickView 1.3 (Flir systems Inc., Burlington, ON, Canada). The SC was measured using scrotal tape (Reliabull, Lane Manufacturing, Denver, CO, USA). Wound healing or incision state was assessed using a 5-point scale previously described by Mintline et al. (2014).

Stride length was collected as previously described by Meléndez et al. (2017*a*). Calves were recorded walking on day −1, 7, and weekly thereafter until day 42 to measure the stride length of the back legs of each calf from pictures captured with GOM player (GOM Lab, Gretech Corporation, Seoul, South Korea) and measured using Image J (National Institute of Health Image, Bethesda, MD, USA).

On day −1, all calves were fitted with an accelerometer data logger (HOBO Pedant G; Onset Computer Corp., Pocasset, MA, USA) to measure standing and lying time (total time standing or lying during the day, %), standing and lying duration (mean standing and lying bout duration, min), and standing and lying bouts (number; *n*) (UBC AWP 2013). Accelerometers were wrapped with plastic to protect them from moisture and covered with a foam pad to protect the animals from abrasions and were attached between the hook and the fetlock of the hind legs with vet wrap (Professional Preference, Calgary, AB, Canada). The accelerometers were set to log data at 1 min intervals which allowed for approximately 14 d of data to be stored on each device. However, the accelerometers were changed weekly and placed on the opposite leg to minimize lesions over the 42 d study period.

Calves were fitted with penning tags on their backs for individual identification during visual observations of behaviours related to pain. Cameras (2.0 MP Bullet Camera, Avigilon, Vancouver, BC, Canada) were mounted on steel poles 6 m above the pens to record continuously for 8 h d^{-1}. The video recordings from days 7, 15, 22, 29, and 34 were analyzed using Observer XT software (Noldus Information Technology Inc., Leesburg, VA, USA) by two observers, analyzing 4 min of continuous video every 10 min for a 4 h period in a subset of six animals per treatment. Observation time was the same across all data collection days and was conducted relative to when calves were returned to their home pens on day 0 (3 h post treatment). The same subset of calves was observed throughout the study. Inter- and intra-rater reliability were 0.95% and 0.91%, respectively. The behaviours evaluated were suckling, tail flicking, foot stamping, head turning, and lesion licking as described by Meléndez et al. (2017*a*).

Calculations and statistical analyses

The schematic boxplot and proc univariate procedures of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) were used for outlier detection and to test normality. Thus, Hp, SAA, and N:L ratio were transformed to a log-scale, and behavioural data were transformed to root square +1 to achieve a normal distribution prior to statistical analysis. Data from HOBO accelerometers were summarized by weeks.

Data collected on day −1 and day 0 for performance, Hp, SAA, and CBC were averaged and used as a covariate, and for stride length, data used as a covariate were only from day −1.

Animal was the experimental unit. Performance data were analyzed using a mixed-effects model (SAS version 9.4, SAS Institute Inc., Cary, NC, USA). The model included treatment, medication, and their interactions as main effects and pen and animal nested within pen as random effects. Hair cortisol, MST, SC, stride length, and all transformed data described above were analyzed using a mixed-effects model (SAS version 9.4, SAS Institute Inc., Cary, NC, USA) with repeated measures. The model included treatment, medication, time, and their interactions as main effects, and pen and animal within pen as random effects. Time was considered as a repeated factor, and for each analyzed variable, pen and animal within pen (the error term) were subjected to three variance–covariance structures: compound symmetry, autoregressive order 1, and unstructured. The covariance structure that minimized Schwarz's Bayesian information criterion was considered as the most desirable analysis. Significance was established at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

The effect of treatment and medication on the time to achieve each healing score was analyzed with a Wilcoxon–Mann–Whitney test (SAS version 9.4, SAS Institute Inc., Cary, NC, USA) with the exception of

Table 1. Performance of noncastrated, knife, and combination of branding and knife castrated with or without a single application of subcutaneous (s.c.) meloxicam in Angus crossbred calves castrated at 2 mo of age, from day 7 post castration until the end of the study (day 42).

	CN		KN		KB			*P* value						
Items	NM	M	NM	M	NM	M	SEM	Trt	Med	Trt × Med	Time	Trt × time	Med × time	Trt × Med × time
Initial BW (kg)	125	124	134	128	128	130	6.7	0.48	0.73	0.79	—	—	—	—
Final BW (kg)	178	180	172	173	171	173	1.9	<0.001	0.21	0.84	—	—	—	—
ADG (kg d^{-1})	1.17	1.22	1.09	1.07	1.01	1.09	0.046	<0.01	0.36	0.96	0.01	0.61	0.62	0.24
Weaning weight (kg)	230	226	222	220	222	222	5.7	0.02	0.34	0.84	—	—	—	—

Note: CN, noncastrated nor branded but handled as castrated and branded; KN, calves castrated with a Newberry knife without being branded; KB, calves branded with hot iron and castrated with a Newberry knife; NM, single s.c. injection of lactated ring at castration time; M, single injection of s.c. meloxicam (0.5 mg kg^{-1}) at castration time; Trt, procedure effect; Med, medication effect; SEM, standard error of the mean; BW, body weight; ADG, average daily gain (least square means from day 7 to day 42).

CN calves. In addition, the univariate procedure (SAS version 9.4, SAS Institute Inc., Cary, NC, USA) was used to calculate medians and the 95% distribution-free confidence limits. Significance was established at $Z \leq 0.05$ and trends at $0.05 < Z \leq 0.10$.

Results and Discussion

Final BW (day 42) and average daily gain (ADG) were greater ($P < 0.01$) in CN (179 ± 1.4 kg, and 1.2 ± 0.03 kg d^{-1}) than KN (173 ± 1.4 kg, and 1.1 ± 0.03 kg d^{-1}) and KB (172 ± 1.4 kg, and 1.0 ± 0.03 kg d^{-1}) calves. These results are in agreement with previous studies reporting that castration and other painful procedures are known to reduce ADG which has been attributed to inappetence related to pain (Fisher et al. 1996; Earley and Crowe 2002; Weary et al. 2006), as well as suppression of androgens after castration (Steen and Kilpatrick 1995). However, other authors did not observe a reduction in ADG after castration (Fell et al. 1986; Pieler et al. 2013; Webster et al. 2013). According to the quadratic relationship between reduction of gain and castration age described by Bretschneider (2005), calves castrated at 2 mo of age and weighed 42 d post castration should lose approximately 4 kg of BW. In the present study, reduction in gain in KN and KB calves compared with CN calves was 6.5 kg and 7.1 kg, respectively. Differences observed between the reduction of gain calculated according to the equation reported by Bretschneider (2005), and the actual weight loss observed in the present study, could be attributed to the differences in handling, postoperative care, or breed. The lack of differences in weight when castration was performed alone or in combination with branding could be explained by the short-lived effects of branding (Stookey and Watts 2004) or by the poor sensitivity between weight gain and acute pain associated with localized tissue trauma. Similar findings were reported by Schwartzkopf-Genswein et al. (1997*a*) who did not observe reduced gain 10 or 28 d after branding in newly weaned Charolaise-cross steer calves, which they attributed to the fact that the stress and (or) pain of hot-iron branding was not severe or long enough to impair performance. Similarly, Tucker et al. (2014*b*) did not find differences in ADG between branded and non-branded Angus–Hereford crossbred calves; but found that wounds were sensitive to palpation for at least 10 wk after branding. However, even though the brand wounds were still sensitive during palpation, it may not have been severe enough to impair performance. No effect ($P > 0.10$) of medication or treatment × medication was observed on performance data (Table 1). We hypothesized that because meloxicam has a longer half-life compared with other NSAIDs (ketoprofen or flunixin meglumine) (Stock and Coetzee 2015), it may attenuate the detrimental effects of castration on performance that other NSAIDs failed to prevent (Ting et al. 2003*a*, 2003*b*; Webster et al. 2013; Pang et al. 2006, 2008; Petherick et al. 2014). However, results from the present

Table 2. Hair cortisol, haptoglobin, serum amyloid-A, blood cell count, scrotal circumference, and maximum scrotal temperature of noncastrated, knife, and combination of branding and knife castrated with or without a single application of subcutaneous (s.c.) meloxicam in Angus crossbred calves castrated at 2 mo of age, from day 7 post castration and post castration and branding until the end of the study (day 42).

	CN		KN		KB			*P* value					
Items	NM	M	NM	M	NM	M	SEM	Trt	Med	Trt × Med	Time	Trt × time	Med × time	Trt × Med × time
Hair cortisol[a] (pg mL^{-1})	10.2	10.9	11.3	10.4	9.1	11.9	0.16	0.96	0.34	0.49	<0.01	0.11	0.18	0.23
Haptoglobin[a] (g L^{-1})	0.13	0.14	0.14	0.13	0.14	0.13	0.002	0.79	0.78	0.46	<0.01	0.11	0.24	0.46
Serum amyloid-A (μg mL^{-1})	48.4	47.4	44.9	39.9	44.1	43.8	0.12	0.52	0.60	0.87	<0.01	0.31	0.99	0.12
Blood cell count														
WBC	9.9	11.1	11.2	11.7	11.3	10.3	0.76	0.44	0.71	0.35	<0.01	0.20	0.15	0.62
Platelet	537	501	532	502	511	517	29.3	0.98	0.35	0.70	0.22	0.82	0.60	0.18
N:L ratio	0.57	0.60	0.51	0.57	0.49	0.54	0.17	0.68	0.21	0.87	<0.01	0.65	0.04	0.85
Scrotal circumference (cm)	14.1ab	14.5a	14.3ab	13.3c	13.6bc	14.3ab	0.49	0.41	0.92	0.07	<0.01	<0.01	0.92	0.52
Maximum scrotal temperature (°C)	36.2	36.3	36.2	36.3	36.4	36.1	0.26	0.98	0.74	0.39	<0.01	0.46	0.43	0.97

Note: Means within a column not sharing a lowercase letter differ significantly at the $P < 0.05$ level. CN, noncastrated nor branded but handled as castrated and branded; KN, calves castrated with a Newberry knife without being branded; KB, calves branded with hot iron and castrated with a Newberry knife; NM, single s.c. injection of lactated ring at castration time; M, single injection of s.c. meloxicam (0.5 mg kg^{-1}) at castration time; Trt, procedure effect; Med, medication effect; SEM, standard error of the mean; WBC, white blood cells; N:L, neutrophil to lymphocyte ratio.

[a]Data transformed to Napierian logarithm. Data presented herein are least square means without transformation, SEM, and *P* values form transformed values.

study showed that a single s.c. injection of meloxicam did not have an effect on ADG in knife-castrated calves with or without being hot-iron branded over 42 d. In addition, the lack of differences in treatment × time effects 7 d post castration, and the low levels of testosterone in 2-mo-old calves (Amann and Walker 1983), suggest that pain suffered at the time of and for the first few days after knife castration, independent of whether the calves were branded or not, might be the main cause of the reduction in ADG observed in this study as opposed to reduced testosterone levels.

No differences in treatment, medication, treatment × medication, or their interactions with time ($P > 0.10$) were observed for hair cortisol concentrations (Table 2). Similarly, Marti et al. (2017*a*) did not observe differences in hair cortisol concentrations between noncastrated and knife-castrated calves at 2 mo of age on day 35 suggesting that the pain experienced by knife-castrated calves might not be intense or long enough to see differences after castration. However, it was expected that the combination of castration and branding would increase the deposition of cortisol within the hair. This hypothesis was based on the findings of Tucker et al. (2014*a*, 2014*b*) who reported greater pain sensitivity values (g force) in hot-iron-branded calves using a Von Frey anesthesiometer applied to the brand site for at least 10 wk after the procedure. However, as discussed previously, pain sensitivity of the brand site might not be sufficiently intense to result in increased hair cortisol concentrations, or pain sensitivity may be poorly correlated with other indicators of stress and (or) pain. For example, plasma cortisol was shown to be greater in branded compared with nonbranded calves for up to 40 min after the procedure (Schwartzkopf-Genswein et al. 1997*c*), in contrast no differences were observed in the reluctance to enter into the handling facility between branded compared with nonbranded calves (Schwartzkopf-Genswein et al. 1997*a*). In addition, the lack of treatment differences in ADG, hair cortisol, and acute phase proteins (APP) may indicate that the greater pain sensitivity of the brand site observed by Tucker et al. (2014*b*) at the time of palpation does not necessarily mean that the calves experienced continual pain, but rather it was only experienced for short periods of time during palpation.

The absence of medication effect on hair cortisol concentrations in this study was in contrast with the findings of Creutzinger et al. (2017), who reported that surgically castrated calves that did not receive meloxicam had 13.8% greater concentrations of hair cortisol than noncastrated calves, and 7.8% greater concentrations of hair cortisol than surgically castrated calves provided s.c. meloxicam at the time of castration in samples collected 14 d post procedure. Differences between the present study and Creutzinger et al. (2017) could be due to differences in sampling times between experiments. Hair cortisol reflects the retrospective circulation of cortisol levels during hair growth (Meyer and Novak 2012),

therefore, the longer the growth of the hair shaft post castration the smaller the proportion of castration-related cortisol deposited within a sample. This may explain why Creutzinger et al. (2017) did observe treatment differences 14 d post castration, whereas no differences were observed between treatments in the present study where samples were collected on day 42 possibly diluting the total castration-related cortisol concentrations within the hair shaft.

When infection, inflammation, or tissue trauma occurs, the acute-phase reaction is activated, which involves behavioural, physiological, biochemical, and nutritional changes (Ceciliani et al. 2012). During this reaction, APP production is increased to restore homeostasis (Murata et al. 2004). Two of the most common innate immune APPs are Hp and SAA, and their circulating concentrations are related to the extent that the tissue is damaged (Murata et al. 2004). Therefore, an increase in Hp and SAA concentrations following knife castration and hot-iron branding would be expected. However, no differences ($P > 0.10$) were observed in either Hp or SAA concentrations associated with treatment, medication, treatment × medication, or their interactions with time. Lack of differences in the APP response might be due to suboptimal sampling times. For example, Meléndez et al. (2018*b*) observed that SAA concentrations were elevated during the first 3 d after castration and branding were conducted. Brown et al. (2015) found differences in Hp up to 72 h post castration and Warnock et al. (2012) up to day 6 post castration in weaned calves.

Complete blood cell counts were found to be within the normal ranges for WBC, platelet count, and N:L ratio reported for cattle of similar age (Jones and Allison 2007), although an effect of medication × time was observed for N:L.

Despite the lack of differences in APP concentrations, a treatment × time effect ($P < 0.001$) was observed in SC (Table 2). Scrotal circumference for KN and KB calves was greater than CN on day 7 and day 14 post castration as a result of the inflammation caused by surgical castration (Molony et al. 1995; Stafford et al. 2002; Marti et al. 2017*a*). Mintline et al. (2014) observed that inflammation peaked 2–3 d post surgical castration, whereas Robb and Wood (1990) and Marti et al. (2017*b*) observed wound swelling up to 14 d after the procedure. In addition, SC of KB calves tended ($P = 0.08$) to be greater than KN calves the first 14 d post procedure. The inflammatory response is the first phase of the complex wound healing process, which causes changes in the composition of immune cells in tissues, cell responsiveness to inflammatory stimuli, and regulation of signaling pathways (Medzhitov and Horng 2009). During the inflammatory response, pro-inflammatory factors are released into the local wound site with the goal to re-establish tissue integrity and homeostasis (Abdullahi et al. 2014). Consequently, differences between SC in KN and KB calves might be explained by the fact that KB calves had two wound sites (scrotal and branding wounds) that initiated the inflammatory process, which may have decreased the response of the inflammatory phase at each individual wound site because there was more than one wound to heal. Scrotal circumference did not differ ($P > 0.10$) between KN and KB calves, but it was smaller than in CN calves between day 21 and day 42 post castration which may be explained by the fact that surgical wounds were observed to reduce in size as inflammation decreased and wound healing occurred (Mintline et al. 2014), whereas CN calves increased their scrotal size as the testicles developed (Coulter and Foote 1979), which may also explain the lack of differences ($P = 0.98$) observed in MST in this study (Table 2). Moreover, a tendency between treatment × medication ($P = 0.07$) was observed for SC; a single s.c. administration of meloxicam tended to reduce ($P = 0.07$) the SC in KN–M calves compared with KN–NM calves, but this effect was not observed when knife castration was combined with branding (Table 2). Mintline et al. (2014) did not observe differences in scrotal size measured with calipers when a single dose of flunixin meglumine was administered after surgical castration.

Following the inflammatory phase, the healing process continues with the proliferation and remodeling phases (Boughton et al. 2006), which in the case of knife castration lasts between 28 and 77 d (Mintline et al. 2014; Norring et al. 2017; Marti et al. 2017*a*). In agreement with Mintline et al. (2014), dramatic changes in healing scores were observed between day 14 and day 28 post procedure, although no differences ($Z > 0.10$) relative to the time of knife castration were observed between KN and KB calves in the present study (Table 3). Furthermore, Mintline et al. (2014) found that wound healing scores did not differ among knife-castrated calves administered flunixin meglumine or not. Similarly, in this study, no differences were observed in healing score between M and NM calves over the 42 d study period (Table 4). The therapeutic effect of s.c. meloxicam would be present during the first phase of wound healing (inflammatory phase) as its half-life is known to be 22 h (Stock and Coetzee 2015). However, as observed by Coetzee et al. (2012), the anti-inflammatory properties of oral meloxicam have been shown to reduce morbidity in weaned castrated beef calves suggesting that its effect may last several days after a single treatment. The inflammatory phase lasted for up to 14 d in this study, and contrary to what was expected, the therapeutic effect of s.c. meloxicam was likely too short lived to cover the entire inflammatory phase. Further research should focus on evaluating repeated injections of s.c. meloxicam during the first 14 d after knife castration to reduce the inflammatory phase of wound healing, as this phase is the most susceptible to infections that can develop into chronic wounds (Harper et al. 2014).

Table 3. Healing score of knife and combination of branding and knife castration in Angus crossbred calves castrated at 2 mo of age, from day 7 post castration and post castration and branding until the end of the study (day 42).

	KN			KB			
Score	*n*	Median	95% FCL	*n*	Median	95% FCL	Trt
1	23	7	7	24	7	7	0.99
2	18	14	14–28	20	17.5	14–28	0.67
3	20	28	21–35	23	28	21–35	0.15
4	19	35	28–42	19	35	28–42	0.11
5	19	42	28–42	17	42	35–42	0.34

Note: KN, calves castrated with a Newberry knife without being branded; KB, calves branded with hot iron and castrated with a Newberry knife; Trt, procedure effect; *n*, number of animals observed at each score; Median, median days to reach each healing score relative to days of castration and castration and branding; FCL, 95% confidence limits distribution free of the days to reach each healing score.

Table 4. Healing score of nonmedicated and medicated with a single application of subcutaneous (s.c.) meloxicam in Angus crossbred calves castrated at 2 mo of age, from day 7 post castration and post castration and branding until the end of the study (day 42).

	NM			M			
Score	*n*	Median	95% FCL	*n*	Median	95% FCL	Med
1	24	7	7	23	7	7	0.99
2	20	14	14–28	18	21	14–28	0.31
3	21	28	21–35	22	28	21–35	0.97
4	18	35	28–42	20	35	28–42	0.90
5	19	42	35–42	17	42	35–42	0.57

Note: NM, single s.c. injection of lactated ring at castration time; M, single injection of s.c. meloxicam (0.5 mg kg^{-1}) at castration time; Med, medication effect; *n*, number of animals observed at each score; Median, median days to reach each healing score relative to days of castration and castration and branding; FCL, 95% confidence limits distribution free of the days to reach each healing score.

Pain in animals needs to be measured indirectly through assessment of physiological responses (Millman 2013), as animals are unable to communicate their experiences in the same way as humans (Anil et al. 2002). However, physiological parameters may not always be reliable indicators of pain, as they can be confounded with other stressors such as handling, sampling, disease, or circadian rhythms (Weary et al. 2006). Consequently, it is important to detect changes in their normal behaviour, such as an increase in the number of steps measured using pedometers (Currah et al. 2009), increase in standing duration measured using accelerometers (White et al. 2008; Devant et al. 2012; Marti et al. 2017*a*; Meléndez et al. 2017*a*), or changes in normal time budgets assessed via visual observation (Molony et al. 1995; Marti et al. 2010, 2017*a*; Meléndez et al. 2017*a*); all of which have previously been found to be useful in assessing acute and inflammatory pain after castration. No treatment differences ($P > 0.10$) were observed for stride length, standing and lying duration, or standing and lying bouts. A treatment effect ($P < 0.01$) was observed for lying time (Table 5). Overall, CN calves spent less time lying (59.7% ± 0.72%) ($P < 0.01$) than KN (61.7% ± 0.72%) and KB (60.7% ± 0.71%) calves, and KN calves tended ($P = 0.06$) to spend more time lying than KB calves. However, lying time after surgical castration (or in combination with branding) must be used with caution as differences among treatments were <1 h per day. In addition, the present study found an increase in the amount of time calves spent lying in the days after surgical castration, which was the opposite of that observed in other studies where a decrease in lying time during the hours and days after castration were observed (Ting et al. 2003*a*; Devant et al. 2012; Meléndez et al. 2017*a*). When the evaluation extended beyond 7 d, Molony et al. (1995) and Marti et al. (2017*a*) did not find changes in lying time in 1 wk and 2-mo-old calves compared with noncastrated calves. Furthermore, Tucker et al. (2014*b*) reported that the time branded calves spent lying was not different from nonbranded calves.

Table 5. Stride length and behaviour of noncastrated, knife, and combination of branding and knife castrated with or without a single application of subcutaneous (s.c.) meloxicam in Angus crossbred calves castrated at 2 mo of age, from day 7 post castration and post castration and branding until the end of the study (day 42).

	CN		KN		KB			*P* value						
Items	NM	M	NM	M	NM	M	SEM	Trt	Med	Trt × Med	time	Trt × time	Med × time	Trt × Med × time
Stride length (cm)	43.2	42.8	44.0	43.0	42.5	44.3	0.94	0.82	0.87	0.29	<0.01	0.69	0.81	0.59
Lying (%)	59.5	59.6	62.2	61.1	60.4	61.3	0.78	<0.01	0.87	0.11	<0.01	0.13	0.78	0.55
Standing duration (min)	50.8	50.5	49.1	48.9	47.5	50.2	0.20	0.51	0.62	0.62	<0.01	0.89	0.77	0.42
Lying duration (min)	52.0	59.3	63.5	58.3	59.9	64.4	0.15	0.68	0.50	0.12	<0.01	0.29	0.78	0.47
Standing bouts (No.)	12.4	12.5	11.9	12.2	12.7	12.1	0.05	0.48	0.78	0.35	<0.01	0.50	0.17	0.14
Lying bouts (No.)	15.7	16.3	15.3	16.7	15.5	15.1	0.09	0.61	0.40	0.45	<0.01	0.52	0.52	0.41
Suckling[a] (min)	1.7	1.8	1.4	1.5	1.4	1.4	0.05	0.07	0.61	0.83	<0.01	0.99	0.13	0.69
Tail flick[a] (No.)	81.5	81.9	76.8	77.7	78.4	70.9	0.89	0.75	0.91	0.93	0.55	0.73	0.75	0.47
Foot stamping[a] (No.)	2.0	2.7	2.5	2.3	2.3	1.8	0.15	0.87	0.89	0.76	<0.01	0.85	0.18	0.99
Head turning[a] (No.)	7.4	6.5	6.2	6.2	5.7	7.0	0.15	0.70	0.62	0.33	0.19	0.96	0.56	0.65
Lesion licking[a] (No.)	0.3	0.6	0.4	0.5	0.7	0.5	0.07	0.63	0.68	0.53	0.19	0.20	0.24	0.63

Note: CN, noncastrated nor branded but handled as castrated and branded; KN, calves castrated with a Newberry knife without being branded; KB, calves branded with hot iron and castrated with a Newberry knife; NM, single s.c. injection of lactated ring at castration time; M, single injection of s.c. meloxicam (0.5 mg kg^{-1}) at castration time; Trt, procedure effect; Med, medication effect; SEM, standard error of the mean.

[a]Data transformed to root square +1. Data presented herein are least square means without transformation, SEM, and *P* values form transformed values. Behaviours were observed in intervals of 4 min every 10 min over a 4 h period.

Tucker et al. (2014*b*) observed a reduction on lying time on the day that calves were branded in animals administered flunixin meglumine regardless if they were branded or not. In contrast, no effect of medication or treatment × medication on standing or lying time, duration or bouts were observed in the present study from day −1 to day 42. Furthermore, no differences ($P > 0.10$) were observed for pain-related behaviours such as tail flicks, foot stamps, head turns, or lesion licks for treatment, medication, treatment × medication, or their interactions with time, and the treatment tendency ($P = 0.07$) observed for suckling behaviour might not be biologically relevant as differences were <1 min among treatments (CN: 1.8 ± 0.04 min in 96 min observed; KN: 1.5 ± 0.03 min in 96 min observed; and KB: 1.4 ± 0.03 min in 96 min observed). Suckling behaviour has also been used as indicator of pain after castration. Molony et al. (2002) observed that teat seeking was reduced in lambs 2 d after castration, whereas Hay et al. (2003) found that suckling behaviour was reduced in piglets 5 d after castration. Similarly, Meléndez et al. (2017*a*) reported a reduction in eating duration, which included suckling 7 d after knife castration in 2-mo-old calves. Differences in suckling duration were observed by Molony et al. (2002), Hay et al. (2003), and Meléndez et al. (2017*a*) shortly after the procedure (within 3 h).

Conclusion

In conclusion, a single s.c. injection of meloxicam at the time of knife castration or when knife castration was combined with hot-iron branding did not reduce inflammation or improve wound healing between 7 and 42 d after the procedure. In addition, calves exposed to multiple procedures (knife castration conducted in combination with hot-iron branding) did not have reduced ADG, greater or more prolonged concentrations of APP or hair cortisol compared to calves exposed to a single procedure (knife castration) nor did they have reduced rates of wound healing. However, the inflammatory response measured via SC, and time spent standing was greater in calves that were both castrated and branded, suggesting that discomfort might be greater in calves receiving both procedures together as opposed to those receiving each procedure alone.

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